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Redox Proteomics Identification of Oxidatively Modified Brain Proteins in Alzheimer's Disease and Mild Cognitive Impairment: Insights into the Progression of this Dementing Disorder

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Abstract. Alzheimer disease is a common age-related neurodegenerative disease characterized pathologically by senile plaques, neurofibrillary tangles, synaptic disruption, and progressive neuronal deficits. The senile plaques contain amyloid-β (1–42) and amyloid-β (1–40), that has been shown by a number of laboratories to induce oxidative stress and as well as neurodegeneration, although the exact mechanisms remained to be defined. Our laboratory showed an increased oxidative stress in AD and MCI

gen species, and neurotoxic products [54,125]. Several lines of evidence have shown that the NF-κB in microglia is activated by β-amyloid [30,67]. Further, activation of NF-κB can stimulate increased expression of TNF-α, IL-1, IL-6, NOS etc [1,33], that could eventually lead to increased modification of the proteins. However, in the absence of additional research, the exact role of inflammation in AD pathogenesis is unclear.

Further mutations of the genes for presenilin-1 (PS-1), presenilin-2 (PS-2) and amyloid precursor protein (APP) have been observed in inherited AD.

tide, Aβ (40-1), which is non-neurotoxic, forms a Tyr free radical. This latter is an absolute requirement by those who propose Cu(II) binding and reduction as the source of the oxidative stress and neurotoxic properties of Aβ [56].

In addition, Aβ (1–42) can bind to receptors on neuronal and glial cells, e.g., the α7-nicotinic acetylcholine receptor, neurotrophin p75 receptor, the N-methyl-D-aspartate receptor, the receptor for advanced glycation end products (RAGE) [121,123], and others, forming calcium and potassium channels in cell membranes [5,41], decreasing glucose transport across brain endothelial cells [7], and activating the release of chemokines [42] and cytokines [1]. In the present review we discuss protein oxidation and lipid peroxidation in AD brain.

Aβ exist in various forms, i.e., monomer, oligomers and fibrils. But the form of Aβ that is the toxic species is still largely unknown. Our laboratory used *C. elegans* strains (CL 1175 and CL 4176) as an *in vivo* model to test Aβ associated toxicity. Oxidative stress occurred at 24 h of *in vivo* production of human Aβ(1–42) but no fibrillar Aβ was found [40]. The results of this study are consistent with the notion that the oligomeric form of Aβ is associated with oxidative stress in *in vivo* conditions.

Oxidative stress may cause reversible and/or irreversible modifications on sensitive proteins leading to structural, functional and stability modulations [84, 106]. Protein modifications are generally associated with loss of function and may lead to either the unfolding and degradation of the damaged proteins, or aggregation leading to accumulation as cytoplasmic inclusions, as observed in age-related neurodegenerative disorders [35]. Oxidized proteins are highly sensitive to proteolytic degradation by the proteasome [46, 107]. The increase in the level of oxidized proteins in AD brain is associated with loss of the activity of the 20S proteasome, which represents a major enzyme for the degradation of oxidized proteins [61,89,108, 116]. However, a recent study has questioned these findings [44]. Other studies have shown that prolonged oxidized proteins are more resistant to degradation by the 20S proteasome [96,100].

Previous investigations have used immunoprecipitation techniques to identify specific protein targets of oxidation. This procedure is labor-intensive and time-consuming and requires a good guess to the identity of the protein at the beginning. That is, this approach requires a prior knowledge of the protein so the correct antibody for the protein of interest can be used. We used this approach in initial studies to show that creatine kinase (CK) is oxidatively modified in AD brain [2]. CK was already reported to show a diminished activity in AD brain [50]. Further, posttranslational modification of proteins can sometime alter the structure of proteins [109], which could then prevent the forma-

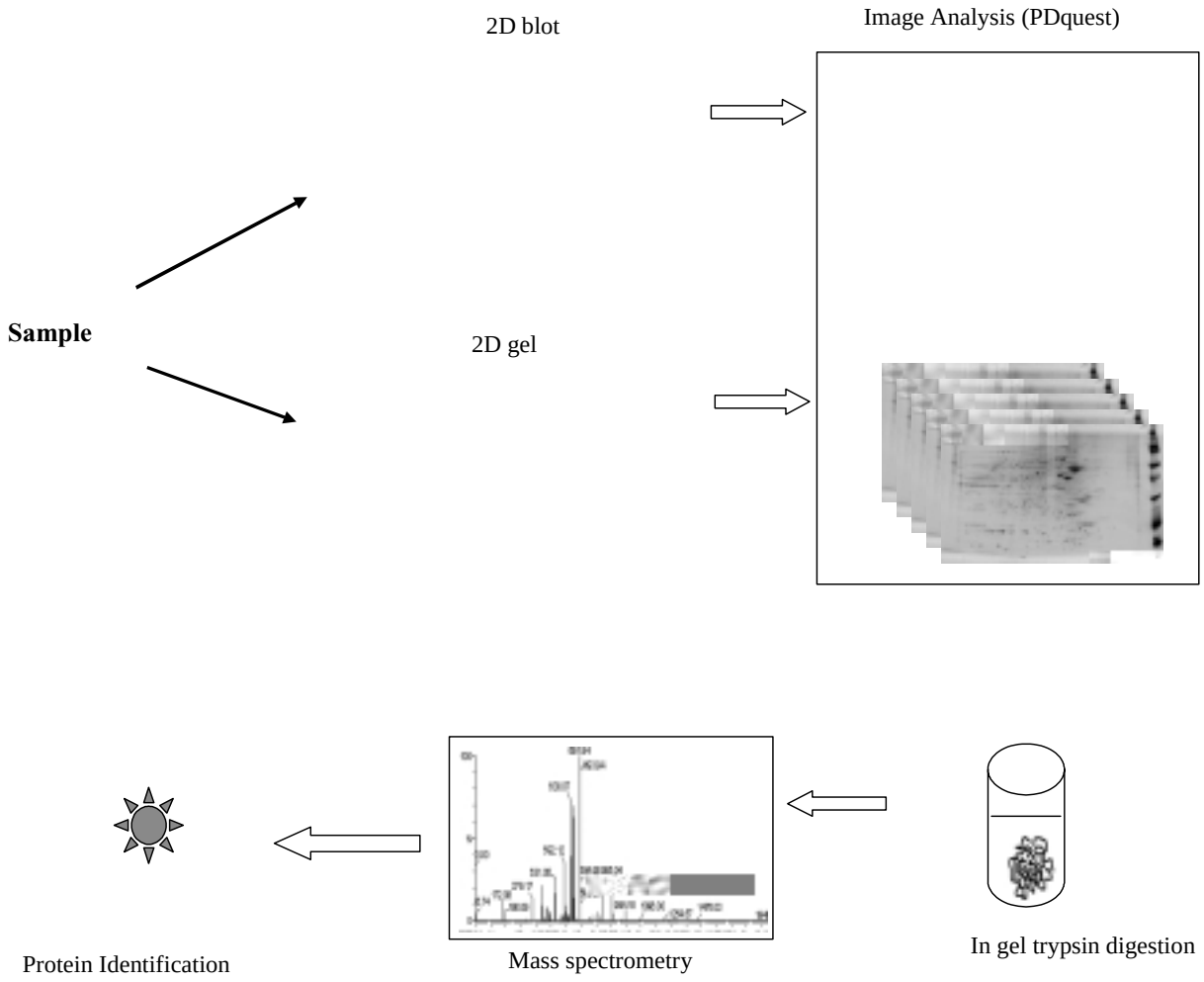
2D blot
Image Analysis (PDquest)
Sample
2D gel
Protein Identification
Mass spectrometry
In gel trypsin digestion

Table 1
Oxidatively modified proteins identified in AD and MCI brain using redox proteomics. Oxidized proteins found in common between AD and MCI brain are indicated in bold

Functions	AD	MCI
Energy dysfunction	CK, **Enolase**, TPI, PGM1, LDH, GAPDH, ATP synthase alpha, **Enolase** **VDAC**	
Excitotoxicity	EATT2, **GS**	GS
Proteasomal dysfunction	UCH L1, HSC 71	–
Lipid abnormalities and Cholinergic dysfunction	Neuropoly-peptide h3	–
PH buffering and CO_2 transport	CA 2	–
Neuritic abnormalities	DRP2, β-actin	–
Tau hyperophosphoryaltion/ Aβ production/ mitosis	**Pin 1**	**Pin 1**
Synaptic abnormalities and LTP	γ-SNAP	–
Pyruvate Kinase M2	–	Pyruvate Kinase M2

AD = Alzheimer's disease, MCI = Mild cognitive impairment, CK = Creatine kinase BB, TPI = Triose phosphate isomerase, PGM1 = Phosphoglycerate mutasepho-SNns5zheimer'e123.468 MCI

Table 2
Fold-increase of carbonylated proteins in AD IPL, AD Hippocampus, and MCI hippocampus relative to respective control brain regions

Carbonylated proteins	Fold-increase in Oxidation	References
AD-Inferior Parietal Lobule		
DRP-2	4.89 ± 0.52	27
$\alpha-$enolase	3.21 ± 0.18	27
Heat shock cognate-71	2.24 ± 0.63	27
Creatine kinase BB	4.89 ± 0.52	26
Glutamine synthase	3.21 ± 0.18	26
Ubiquitin carboxyl-terminal hydrolase L-1	2.24 ± 0.63	26
AD-Hippocampus		
Peptidyl prolyl *cis/trans* isomerase 1	1.36 ± 0.55	112, 111
DRP-2	1.26 ± 0.45	112
Phosphoglycerate Mutase 1	212.30 ± 266.8	112
Carbonic anhydrase	3.27 ± 0.85	112
Enolase 1	2.55 ± 0.62	112
Triose phosphate isomerase	6.44 ± 2.28	112
Gamma-SNAP	3.15 ± 132	112
UCHL-1	2.10 ± 0.45	112
MCI-Hippocampus		
Enolase 1	3.5	21
Glutamine synthetase	4.0	21
Pyruvate kinase M2	3.0	21
Peptidyl prolyl cis/trans isomerase	5.0	21

mal dysfunction and AD [26,112]. In addition, UCH L-1 rescues Aβ-induced decreased synapatic function and contextual memory [104], suggesting that oxidatively dysfunctional UCH L-1 could contribute to the known memory defects in AD.

Lipid Abnormalities And Cholinergic Dysfunction

In AD brain neuropolypeptide h3, a phosphatidylethanolamine binding protein [PEBP] or hippocampal cholinergic neurostimulating peptide [HNCP], has been identified as a specifically oxidized protein [28]. PEBP plays an important role in maintaining phospholipid asymmetry, which is important to maintain the structure and function of membranes [29,81]. The oxidation of this protein could lead to the loss of PEBP activity that may lead to loss of membrane asymmetry, which, in turn, may initiate apoptosis and consequently to cell death. Our laboratory showed that the addition of Aβ (1–42) and HNE to synaptosomes lead to loss of phospholipid asymmetry [81]. This enzyme also regulates the levels of choline acetyltransferase, an enzyme that is reported to have decreased activity in AD brain [63], and this could be related to the reported cognitive decline in AD.

Neuritic Abnormalities

Dihydropyrimidinase related protein 2 (DRP-2), and β-actin are structural proteins that are found to be oxidized in AD brain [27,112]. DRP2 is normally expressed in developing brain and found only sparingly in adult brain. The oxidation of actin could be related to the loss of cytoskeletal network integrity and activation of cellular events that may lead to apoptosis. The oxidation of DRP-2 could impair interneuronal communication and repair and also interfere with the regulation of the activity of collapsin, a protein that is involved in dendritic elongation and pathfinding [47,60]. In AD brain, oxidation of these proteins could be related to the observed shortened dendritic length [32] and cognitive impairment in AD [57].

Tau Hyperphosphorylation/Aβ Production/Prevention Of Exit Of Neurons From Mitosis

Peptidyl-prolyl cis/trans isomerase (Pin 1) was found to be one of the oxidatively modified proteins in AD hippocampus with an associated decrease in enzyme activity [111,112]. This protein binds to a phosphorylated serine or threonine on the N-terminal side of a proline of target proteins. Pin 1 catalyzes the conversion of the *cis* to *trans* conformation and vice versa of the proline in target proteins, thereby conformationally regulating target protein activity. Pin 1 regulates activity of protein phosphatase 2A (PP2A), which dephosphorylates tau, and GSK-3β, which phosphorylates tau. Recent studies show that Pin1 is colocalized with phosphorylated tau and also shows an in inverse relationship between expression of tau and Pin 1 in Alzheimer's tautopathies [70]. Pin 1 also modulates Aβ production by regulating APP, thereby keeping the Aβ levels low [87]. Pin 1 protein also prevents neu-

Table 3
Fold-increase of nitrated proteins in AD IPL and Hippocampus relative to respective control brain regions

Nitrated proteins	Fold-increase in Oxidation	References
AD-Inferior Parietal Lobule		
$\alpha-$Enolase	3.12 ± 0.87	28
Triosephosphate isomerase	4.8 ± 2.09	28
Neuropolypeptide h3	7.65 ± 3.71	28
$\beta-$Actin	1.44 ± 0.68	28
l-Lactate dehydrogenase	1.62 ± 1.18	28
$\gamma-$Enolase	1.53 ± 1.11	28
AD-Hippocampus		
Alpha- Enolase	3.47 ± 0.90	114
Carbonic anhydrase II	2.53 ± 0.72	114
Glyceraldehyde-3-phosphate dehydrogenase	2.18 ± 0.64	114
ATP synthase alpha chain	3.26 ± 1.70	114
Voltage-dependent anion-channel protein-1	5.11 ± 1.20	114

ronal cells from exiting mitosis. Therefore, oxidatively dysfunctional Pin 1 may be critically important in the known major pathologies of AD, i.e., hyperphosphorylation of tau (NFT), increased production of Aβ (SP), and loss of neurons or synapses due to cell cycle machinery failure [19,70,87,105].

Synaptic Abnormalities And LTP

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